Bioresource Technology 101 (2010) S75–S77

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Comparison of several methods for effective lipid extraction from microalgae

Jae-Yon Lee, Chan Yoo, So-Young Jun, Chi-Yong Ahn, Hee-Mock Oh *

Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 111 Gwahangno, Yuseong-gu, Daejeon 305-806, Republic of Korea

ARTICLE INFO

Article history:
Received 7 November 2008
Received in revised form 16 March 2009
Accepted 19 March 2009
Available online 21 April 2009

Keywords:
Biodiesel
Cell disruption
Lipid extraction
Microalgae

ABSTRACT

Various methods, including autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution, were tested to identify the most effective cell disruption method. The total lipids from *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were extracted using a mixture of chloroform and methanol (1:1). The lipid contents from the three species were 5.4–11.9, 7.9–8.1, 10.0–28.6, 6.1–8.8, and 6.8–10.9 g L^{-1} when using autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution, respectively. *Botryococcus* sp. showed the highest oleic acid productivity at 5.7 mg L^{-1} d^{-1} when the cells were disrupted using the microwave oven method. Thus, among the tested methods, the microwave oven method was identified as the most simple, easy, and effective for lipid extraction from microalgae.

1. Introduction

Global warming and the exhaustion of fossil fuels are major world-wide problems. Thus, the production of biodiesel using various materials, such as plants, microalgae, and animal fat, has been attempted as an alternative energy source (Vasudevan and Briggs, 2008). In particular, biodiesel has two main advantages, the mitigation of carbon dioxide and as a substitute for petroleum (Chisti, 2008). Plus, microalgae also have certain advantages compared to other energy crops, including a high growth rate, short growth time, high biomass production, and low land use (Milne et al., 1990).

The key processes involved in biodiesel production using microalgae are cultivation, harvest, lipid extraction (cell disruption), and the transesterification of the lipids. Although all these steps are essential, the cell disruption is particularly important, as the contents of the extracted lipids are determined according to the disruption method and device. Therefore, the appropriate cell disruption method and device are key to increasing the lipid extraction efficiency.

Various methods, such as microwaves, sonication, and bead-beating, have already been used for cell disruption. For example, microwaves that shatter cells using the shock of high-frequency waves were recently suggested as an efficient method for vegetable oil extraction (Cravotto et al., 2008; Virot et al., 2008), while sonication that cracks cell wall and membrane due to a cavitation effect has been widely used to disrupt microbial cells (Engler, 1985; Lee et al., 1998). Plus, bead-beating that causes direct mechanical damage to cells based on high-speed spinning with fine beads has been used on both a laboratory and industrial scale (Lee et al., 1998; Geciova et al., 2002). However, the most efficient method for microalgae has not yet been settled. Accordingly, further methods of cell disruption, including autoclaving at a high temperature and pressure and the use of a 10% NaCl solution to break cell wall by osmotic pressure, were compared with microwaves, sonication, and bead-beating to determine the most efficient method.

2. Methods

2.1. Microalgae cultivation and harvest

Botryococcus sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were obtained from the Biological Resource Center (BRC), Korea. The microalgae were incubated in separate 9-L jars in a BG11 medium (Rippka et al., 1979) with 0.3 v/v/m air and 150 μmol m^{-2} s^{-1}. The biomass of cultured cells was harvested by centrifugation, then the wet cell mass was frozen overnight at −70 °C and freeze-dried at −70 °C under a vacuum.

2.2. Cell disruption

An aliquot (0.5 g) of the dry cell biomass was blended with 100 mL of distilled water and the mixture disrupted using five different methods as follows: (1) autoclaving at 125 °C with 1.5 MPa for 5 min, (2) bead-beating using a bead beater (bead diameter

* Corresponding author. Tel.: +82 42 860 4321; fax: +82 42 879 8103.
E-mail addresses: heemock@kribb.re.kr, envbio0720@hanmail.net (H.-M. Oh).

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.03.058

0.1 mm, BioSpec Products Inc., USA) at a high-speed of 2800 rpm for 5 min, (3) microwaves using a microwave oven at a high temperature (about 100 °C and 2450 MHz) for 5 min, (4) sonication using a sonicator (Sonic and Materials Inc., USA) at a resonance of 10 kHz for 5 min, and (5) osmotic shock using a 10% NaCl solution with a vortex for 1 min and maintained for 48 h.

2.3. Lipid extraction

The total lipids were extracted by mixing chloroform–methanol (1:1 v/v) with the samples in a proportion of 1:1 using a slightly modified version of Bligh and Dyer's method (1959). The mixtures were transferred into a separatory funnel and shaken for 5 min. The lipid fraction was then separated from the separatory funnel and the solvent evaporated using a rotary evaporator. The weight of the crude lipid obtained from each sample was measured using an electronic scale.

2.4. Fatty acid composition analysis

A fatty acid composition analysis was performed using a gas chromatograph (Shimadzu GC-2010, Japan). Fifty milligram samples were placed into capped test tubes, saponified with 1 ml of a saturated KOH–CH_3OH solution at 75 °C for 10 min, and then submitted to methanolysis with 5% HCl in methanol at 75 °C for another 10 min. Thereafter, the phase containing the fatty acids was separated by adding 2 ml of distilled water and then recovered. The components were identified by comparing their retention times and fragmentation patterns with those for standards (Xu et al., 2001). Six fatty acids (C16:1, C17:0, C18:0, C18:1, C18:2, and C18:3) were used as the standard materials.

2.5. Statistical analysis

The extracted lipid contents from the three microalgal species were compared according to the five disruption methods using a one-way ANOVA and Tukey test. The level of significant difference was at $P < 0.05$.

3. Results and discussion

3.1. Cell biomass

The three microalgal species were incubated until a similar biomass was produced. While *Botryococcus* sp. was cultured for 14 days, *C. vulgaris* and *Scenedesmus* sp. were only incubated for 7 days (Table 1). The *C. vulgaris* showed the highest biomass productivity at 74.2 mg L^{-1} d^{-1} on day 7, when compared to the other species. The biomass productivity of *Scenedesmus* sp. was similar to that of *C. vulgaris*. However, the biomass productivity of *Botryococcus* sp. was only 35.7 mg L^{-1} d^{-1} which was about half of that of *C. vulgaris* and *Scenedesmus* sp.

3.2. Comparison of lipid extraction methods

The lipid content from *Botryococcus* sp. was about 160.3 mg L^{-1}, which was about 2 times higher than that from *C. vulgaris* and *Scenedesmus* sp. Meanwhile, *C. vulgaris* and *Scenedesmus* sp. showed a similar lipid content. The lipid productivity of *Botryococcus* sp. was highest at 11.5 mg L^{-1} d^{-1}, which was similar to that of *C. vulgaris* (Table 1). A higher lipid content was extracted from the three species when using the microwave oven method rather than the other methods (Fig. 1). The bead-beating and microwave oven methods were the most efficient among the compared methods at 28.1% and 28.6%, respectively, for *Botryococcus* sp. ($P < 0.05$), while the sonication method had the lowest efficiency at 8.8%. Plus, in a previous study, the bead-beating method was also shown to extract a higher lipid content from *Botryococcus braunii* than sonication, homogenization, French press, and lyophilization (Lee et al., 1998). However, despite the efficiency of the bead-beating method, it is not easy to scale-up. For *C. vulgaris*, the autoclaving and microwave oven methods showed the highest efficiency, whereas the bead-beating method showed the lower efficiency at 7.9%. For *Scenedesmus* sp., the microwave oven method showed the highest efficiency, while the efficiencies of the bead-beating, sonication, and osmotic shock methods were similar. Although the osmotic shock method is simple and showed similar results to the bead-beating method for *C. vulgaris* and *Scenedesmus* sp. it required a longer treatment time (48 h).

A similar lipid extraction method using microwaves has already been reported for vegetable oils and animal fats (Mahesar et al., 2008; Virot et al., 2008). Plus, this study found that, the microwave oven method was the most simple, easy, and efficient method for the tested microalgae. Furthermore, this lipid extraction method can be easily scaled-up. In conclusion, the microwave oven method was found to be the most applicable for large-scale lipid extraction from a microalgal biomass.

3.3. Fatty acid composition

The major fatty acid composition of the tested microalgae was determined using a GC analysis (Table 2). In a previous report (Knothe, 2008), palmitic, stearic, oleic, and linolenic acid were recognized as the most common fatty acids contained in biodiesel. In the three tested microalgae, oleic acid (C18:1) and linoleic acid (C18:2) were commonly dominant. Oleic acid was higher in *Botryococcus* sp. and *Scenedesmus* sp. at 6.68 and 10.75 mg g^{-1} dw, respectively, while linoleic acid was highest in *C. vulgaris* at 19.79 mg g^{-1} dw. The daily lipid productivity of each microalgal species is shown in Table 1. The productivity of oleic acid calculated based on the daily lipid productivity was highest for *Botryococcus* sp. at 5.7 mg L^{-1} d^{-1}. In particular, oils with a high oleic acid content have been reported to have a reasonable balance of fuel properties (Rashid et al., 2008). The properties of a biodiesel fuel, including its ignition quality, combustion heat, cold filter plugging

Table 1
Biomass, lipid contents, and productivity of *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp.

Item	Algal species		
	Botryococcus sp.	*C. vulgaris*	*Scenedesmus* sp.
Incubation days	14	7	7
Dry weight (g L^{-1})	0.5	0.5	0.5
Biomass productivity (mg L^{-1} d^{-1})	35.7	74.2	71.4
Average lipid content (mg L^{-1})	160.3	77.9	66.5
Lipid productivity (mg L^{-1} d^{-1})	11.5	11.1	9.5
Microwave oven method			
Lipid productivity (mg L^{-1} d^{-1})	10.2	7.4	7.4
Oleic acid productivity (mg L^{-1} d^{-1})	5.7	1.2	4.2

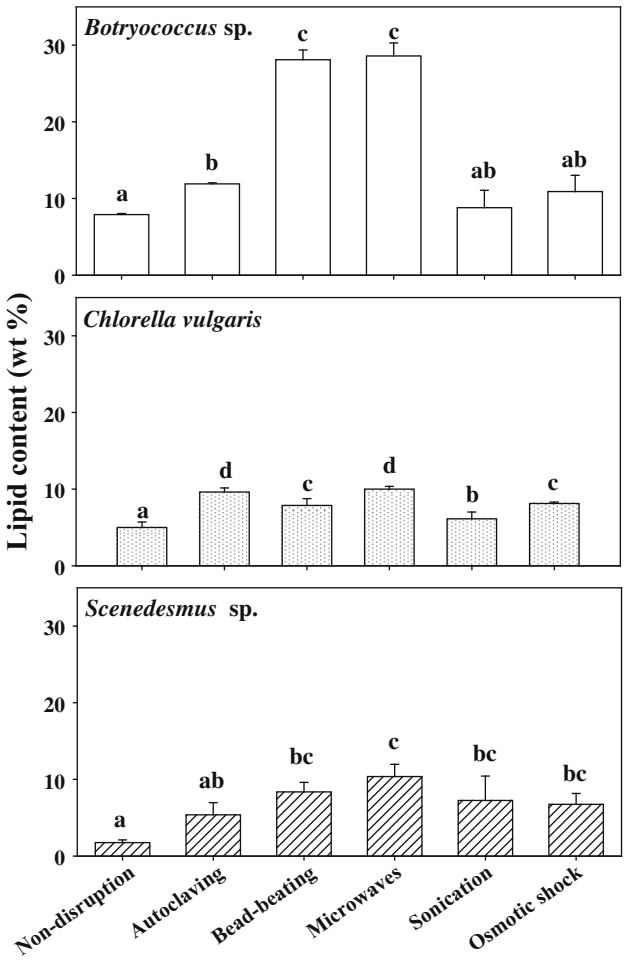

Fig. 1. Lipid extraction efficiency according to species and method. The different letters in the graphs indicate a significant difference at $P < 0.05$.

Table 2
Fatty acid composition of *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp.

Fatty acid	Amounts of fatty acids (mg g^{-1} dw)					
	Botryococcus sp.		*C. vulgaris*		*Scenedesmus* sp.	
C16:1	0.58	(4.8)	ND		ND	
C17:0	0.10	(0.8)	0.20	(0.1)	0.13	(0.7)
C18:0	0.52	(4.3)	0.85	(3.4)	0.57	(3.0)
C18:1	6.68	(55.7)	4.07	(16.3)	10.75	(57.2)
C18:2	4.10	(34.2)	19.79	(79.4)	6.91	(36.8)
C18:3	0.02	(0.2)	0.03	(0.1)	0.40	(2.2)
Total	12.00	(100)	24.94	(100)	18.78	(100)

*ND: not detected.
(): Fatty acid composition (wt %).

point (CFPP), oxidative stability, viscosity, and lubricity, are determined by the structure of its component fatty esters. As such, a higher oleic acid content increases the oxidative stability for longer storage (Knothe, 2005) and decreases the CFPP for use in cold regions (Stournas et al., 1995). Therefore, among the tested microalgal species, *Botryococcus* sp. showed the highest oleic acid content, making it the most suitable for the production of good quality biodiesel.

4. Conclusions

The efficiency of lipid extraction from microalgae was found to differ according to the species and extraction method. The highest lipid content was extracted from *Botryococcus* sp. and was about two-fold higher compared to that from the other species. Plus, the microwave oven method showed the highest efficiency for all the tested species. *Botryococcus* sp. showed the highest oleic acid productivity at 5.7 mg L^{-1} d^{-1}, when the cells were disrupted using the microwave oven method. Thus, it was concluded that the microwave oven method would appear to be the most simple, easy, and efficient method for lipid extraction from microalgae.

Acknowledgements

This research was supported by a grant M102KP010017-08K1601-01710 from the Carbon Dioxide Reduction and Sequestration Research Center, a 21st Century Frontier Program funded by the Korean Ministry of Education, Science and Technology.

References

Bligh, E.G., Dyer, W.M., 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 26, 126–131.
Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., Cintas, P., 2008. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrason. Sonochem. 15, 898–902.
Engler, C.R., 1985. Disruption of microbial cells. In: Moo-Yoong, M. (Ed.), Comprehensive Biotechnology, second ed. Pergamon Press, Oxford, pp. 305–324.
Geciova, J., Bury, D., Jelen, P., 2002. Methods for disruption of microbial cells for potential use in the dairy industry – a review. Int. Dairy J. 12, 541–553.
Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol. 86, 1059–1070.
Knothe, G., 2008. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuel 22, 1358–1364.
Lee, S.J., Yoon, B.D., Oh, H.M., 1998. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. Biotechnol. Tech. 12, 553–556.
Mahesar, S.A., Sherazi, S.T.H., Abro, K., Kandhro, A., Bhanger, M.I., Van de Voort, F.R., Sedman, J., 2008. Application of microwave heating for the fast extraction of fat content from the poultry feeds. Talanta 75, 1240–1244.
Milne, T.A., Evans, R.J., Nagle, N., 1990. Catalytic conversion of microalgae and vegetable oils to premium gasoline, with shape-selective zeolites. Biomass 21, 219–232.
Rashid, U., Anwar, F., Moser, B.R., Knothe, G., 2008. *Moringa oleifera* oil: a possible source of biodiesel. Bioresour. Technol. 99, 8175–8179.
Rippka, R., Deruelles, J., Waterbury, J.B., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1–61.
Stournas, S., Lois, E., Serdari, A., 1995. Effects of fatty acid derivatives on the ignition quality and cold flow of diesel fuel. J. Am. Oil Chem. Soc. 72, 433–437.
Vasudevan, P.T., Briggs, M., 2008. Biodiesel production – current state of the art and challenges. J. Ind. Microbiol. Biotechnol. 35, 421–430.
Virot, M., Tomao, V., Ginies, C., Visinoni, F., Chemat, F., 2008. Microwave-integrated extraction of total fats and oils. J. Chromatogr. A 1196–1197, 57–64.
Xu, N., Zhang, X., Fan, X., Han, L., Zeng, C., 2001. Effects of nitrogen source and concentration on growth rate and fatty acid composition of *Ellipsoidion* sp. (Eustigmatophyta). J. Appl. Phycol. 13, 463–469.